Monograph No. 25

Practical Concepts for Dose Selection in Chronic Toxicity and Carcinogenicity Studies in Rodents

February 1996

ISSN-0773-6347-25

Brussels, February 1996

ECETOC Monograph No. 25

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CONTENTS

SUMMARY

The process for selecting the Highest Dose to be Tested (HDT) in chronic toxicity and carcinogenicity studies in rodents has been reviewed. Current scientific and regulatory guidelines largely agree on general principles but differ or conflict on practical guidance. This arises from various interpretations of the meaning of the abbrevaition MTD - either Maximum Tolerated Dose or Minimally Toxic Dose (abbreviated to MaxTD and MinTD respectively in this report).

Use of a significantly-toxic high dose has resulted in many compounds being identified as carcinogens but showing no excess tumour incidence at doses slightly below those eliciting frank toxicity. In some cases investigation has demonstrated that tumour induction was secondary to the toxicity, rather than an intrinsic property of the compound. For such compounds measures to protect against the toxicity will also protect against development of tumours. Thus the use of excessively - toxic doses in bioassays can result in loss of specificity with no increased benefit to the protection of human health.

In chronic toxicity studies, the object is to detect all toxic effects of dosage over the study period, so that the high dose should induce significant toxicity. The separation of dose-setting for chronic toxicity studies from that for carcinogenicity studies is thus logical.

The process recommended is selection of the highest doses in chronic toxicity and carcinogenicity studies on the basis of prediction from studies of shorter duration but using different criteria for each study type. The chronic toxicity study should be at least 6-months duration but be limited to one year whilst the carcinogenicity study should last for about 2 years in rats and at least 18-months in mice or hamsters. The HDT in the carcinogenicity study should produce only minimal signs of toxicity while the HDT in the chronic toxicity study should elicit frank toxic effects without increased mortality. Limitation of the exposure period for the chronic toxicity study would permit reduction in the numbers of animals per group (because of lower mortality) and allow the introduction of a further group without increasing animal usage. The lower dose levels used in the chronic toxicity study should be the same as in the carcinogenicity study, improving efficiency and interpretation when the studies are conducted simultaneously.

The proposed design allows flexibility. If unexpectedly high mortality occurs at the HDT of the chronic toxicity study the test is not invalidated; the lower HDT of the carcinogenicity study then allows the results to be readily put into context with respect to possible secondary effects. It is predicted that this design will improve the specificity of the carcinogenicity study without significant loss of protection of exposed populations.

The principles for selection of the HDT in both study types are presented, including the possible use of a multiple of the human exposure where this can be adequately predicted. This allows a degree of harmonisation of the principles employed for dose selection with industrial chemicals, agrochemicals food additives and human and veterinary medicines.

It is recognised that there is a dose beyond which bioassays lose specificity and become subject to distortion, so that regulations specify a limit dose. It is recommended that this concept should be extended to include other factors which might further reduce the limit.

To benefit from the changes recommended, adoption by regulatory agencies internationally will be required. The principles are commended to the OECD for more general consideration.

1. INTRODUCTION

"The ultimate objective of any toxicity study on a chemical intended for use as a drug, pesticide, industrial chemical or for other purposes, is to characterize and quantify risks originating from the product, in order to prevent harm to man and animals" (Hapke, 1975). Carcinogenic risk is studied almost exclusively on groups of rodents, administered a substance over most of their life-span. The studies are usually on three dosage groups, the lowest of which is anticipated to be a no-effect level with the mid and high dose providing a dose-response relationship for any effects which develop. Selection of the highest dose level tested (HDT) has proved controversial. Agencies in the US favour administration of the "maximum tolerated dose" (MaxTD in this report) whilst the OECD and EEC favour a "minimally toxic dose" (MinTD). Both of these have been abbreviated to MTD. (To indicate the dual explanation "MTD" has been applied throughout this report.)

Tumours which develop only at excessively toxic dosages may be formed secondarily to the toxic effects and may thus have no relevance at lower doses, at which such effects are absent. A proportion of the compounds so far tested and found to be positive only at the MaxTD, are believed to represent a trivial, or no carcinogenic risk at typical human exposure levels. Most human carcinogens can be identified in animal tests at half the MaxTD or less (Apostolou, 1990) while of the substances showing carcinogenic activity in the US National Toxicology Programme (NTP), one-third to one-half (dependent on statistical considerations) showed no activity at half the MaxTD (Haseman and Lockart, 1994).

For materials of low toxicity the large doses administered may interfere with the normal physiology or nutrition of animals and produce a response which is not indicative of toxic risk at human exposure levels. Similarly, high concentrations of a low toxicity gas in an atmosphere may result in oxygen depletion.

In addition, compounds of low toxicity may overload primary- or secondary-phase metabolism and distort dose-response relationships. Where there is a quantitative and/or qualitative change in metabolites as 'normal' metabolism becomes saturated with increasing dose, assessment of human risk should be made from dosages producing the metabolic route relevant to man; consideration may have to be given to the existence of human population sub-groups with variations in metabolic capability.

These considerations have led to limitation of the highest dose in studies with low toxicity substances. The limit dose is generally beyond the credible level of human exposure but below that causing serious physiological disturbances.

The adoption of a limit dose raises the question of which other factors should be considered in setting the HDT. If the main purpose of a study is to maximise detection of carcinogenic activity, the use of the maximum possible dose may be justified even though specificity may be lost. If the primary purpose is to determine whether there is significant carcinogenic risk at possible human exposure levels, the HDT should be pitched at a level at which there is little or no overt general toxicity; this is of particular importance when high doses of the test material cause changes leading to chronic cellular proliferation.

Although the use of MaxTD and MinTD in animal bioassays has been standard practice for many years, there is no consensus on their definition (c.f. Appendix A), even by regulatory agencies within the same country, resulting in different criteria for the design of studies and interpretation of data from them. The major conflict is between the criteria applied in the EU and by the US-EPA for changes in body-weight gain (c.f. Section 2.6). This has led to confusion between those responsible for design of studies and subsequently, regulatory authorities who evaluate the dose-response effects observed, so that a study acceptable to one agency may be rejected by another. At worst this could lead to performance of different studies for the same substance for various agencies, whereas the aim should be to harmonise requirements internationally and minimise animal use.

The Task Force was given the following terms of reference :

- review the maximum tolerated dose and the concept of limit doses currently used;
- define the practical, scientific and regulatory principles for establishing the highest dose to be used in long-term carcinogenicity studies using various routes of exposure;
- outline a procedure for selecting the highest dose level.

The existing guidelines and literature and other methods which might be used to set the highest test dose have been reviewed. Comments are made on the overall design of studies including the selection of lower dose levels. A procedure has been proposed for selecting the highest dose level for various routes of exposure having due regard for the practical, scientific and regulatory principles involved.

2. PRACTICAL CONSIDERATIONS

2.1 OBJECTIVES OF CHRONIC TOXICITY AND CARCINOGENICITY STUDIES

Chronic toxicity and carcinogenicity are often examined together in one study with one set of dose levels; this complicates the interpretation of the study. A chronic toxicity study aims to identify the chronic toxic effects and to determine dose-response relationships. The HDT is set sufficiently high to demonstrate toxic effects without causing mortality. The objective of a carcinogenicity study is to observe test animals during the major proportion of their life-span for the development of neoplastic lesions. It is important here that the HDT does not reduce significantly the longevity of the animal due to non-neoplastic effects. Carcinogenicity studies should continue for the majority of the life-span as spontaneous tumours generally arise towards the end of the natural life. In the case of chronic toxicity studies, there are advantages in termination and assessment before the onset of major geriatric changes as these obscure compound-related pathology. In practice, additional chronic toxic effects seldom develop in studies after one year (most occur within 6 months) and 6-months or 1-year is often recommended as the duration for a chronic test.

2.2 CURRENT PRACTICES IN DOSE SETTING

Chronic and carcinogenicity studies are designed with knowledge of the dose-responses observed in 28-day or, more usually, sub-chronic (90-day) repeated dose studies. Ideally the HDT selected should maximise the sensitivity of the test without introducing distortions induced by excessive chronic toxicity. The criteria for selecting this dose vary in different guidelines. The current practice with agrochemicals or industrial compounds is to conform to the most stringent guideline, i.e. that of the US-EPA. If the compound is not to be sold in the USA less extreme guidelines are followed.

The lowest dose "should not cause any significant toxicity and should not interfere with the normal growth, development or longevity of the animals". Most guidelines also require the low dose to be not less than 10% of the high dose (OECD, 1981; EEC, 1985; UK-HSC,1982; IARC, 1980; US-EPA (TSCA), 1983). Since most biologically active compounds show toxic activity at 10% of the HDT, the latter recommendation is largely ignored.

The intermediate dose or doses are fixed between the other levels. OECD (1981) and US-EPA (TSCA) (1983) mention the use of toxicokinetic measurements of the compound, if known, in setting the intermediate dose. Most guidelines suggest that the intermediate dose levels should ideally exhibit minimally toxic effects; in practice this is difficult to predict and often the expected NOEL is selected with the lowest dose being used as a 'fall back'. In the US-NTP programme, the mid dose has usually been

half the MaxTD, though this may be unduly restrictive for demonstration of dose response. In selecting the mid- and low dose levels, the NOEL should cover the human exposure level with an adequate safety margin. Additionally, dose levels may be selected to cover any threshold effects or provide evidence of effects at specific dose rates which could be related to human exposure.

A limit test may be performed for low toxicity compounds. Regulations require only a control and limit dose group. In practice chronic toxicity and/or carcinogenicity limit tests are rarely performed since a repeat study will be needed if unexpected effects or statistically significant differences are found. In practice the 'limit dose' may be used as the HDT in a conventional study design.

2.3 ROUTES OF ADMINISTRATION

Dose selection is dependent on the route of exposure.

The physical form or the usual conditions of exposure to compounds often dictate the route of compound administration, e.g., gases and vapours are administered by inhalation, industrial oils by dermal application and pharmaceutical compounds by gavage. In dose setting and interpretation of the results it is important to understand the implications of the different procedures.

2.3.1 Oral Route

Oral administration allows high dose levels with a high probability of good absorption. Oral administration can be by gavage of liquid suspension or solution, or by incorporation into the diet or the drinking water.

Gavage doses can be given in proportion to the body-weight of individual animals, an accurate form of dose administration. The disadvantages are the often rapid absorption of a bolus dose with production of high, short-term blood levels, and the technical resources needed for repeated gavage.

Incorporation into the diet or drinking water is the most practical manner of administration. Each dose group may be given a constant dietary (or water) concentration, in which case the daily dose is higher in young than in fully grown animals but the same formulation and analytical procedures can be used throughout the study. The concentration may also be changed periodically to achieve a reasonably constant dose in proportion to body-weight. The test substance may alter diet or water acceptability and reduce the intake so the target dose may not be achieved. In older animals with a high percentage of adipose tissue and low metabolic capacity in their bodies, increasing the administration rate with body

mass can result in chronic metabolic stress. Thus the MaxTD may be exceeded at a late point in the study when corrective action is likely to be of limited effect.

2.3.2 Inhalation Route

The inhalation route provides less rigorous control of dose rate, is technically more demanding and requires more resources than oral administration. With irritant materials, rodents may modify their breathing pattern to minimise exposure. Some oral exposure is inevitable through grooming and swallowing inhaled material after lung clearance via the muco-ciliary route. An accurate estimate of the retained dose can be extremely difficult to achieve. Inhalation mimics the normal manner of exposure to gases, vapours, dusts, mineral fibres, and some materials (e.g. asbestos minerals, crystalline silica) demonstrate their true risks to man only when administered by this route.

2.3.3 Dermal Administration

Long-term dermal exposure is only used to assess carcinogenicity in the skin. This route suffers disadvantages. The fur may need to be clipped prior to administration with a risk of skin abrasion. Concentrations applied need to be carefully selected to avoid chronic irritation which can confound interpretation of the results. Grooming leads to ingestion and its prevention is practical for only short periods of time; this may determine the pattern of dermal exposure.

2.4 TOXICOKINETICS

The term toxicokinetics refers to the changes in concentration of a compound and/or its metabolite(s) in body fluids and tissues with time. A reliable dose selection would be helped by knowledge of the extent of (systemic) absorption, its time course and the relationship to toxicological and carcinogenic effects. These data can be derived from measurements of the concentration in body fluids in dose- ranging studies and in some instances *in vitro* studies could be useful (ICH, 1993; Anon, 1994). With pharmaceutical compounds toxicokinetic studies are performed in both human beings and experimental animals early in the cycle of toxicity testing. For agrochemicals and industrial chemicals human and animal data are not always available at the time doses for chronic toxicity studies are set. When available, they are of great assistance in defining when metabolic saturation is likely to occur.

Until recently toxicokinetic data were rarely obtained for industrial chemicals. However, emerging EEC legislation will ask for systematic evaluation of toxicokinetic data so that basic information should be available for dose-setting in any chronic or carcinogenicity study. This issue has been comprehensively

addressed (ECETOC, 1992).

2.5 LIMIT DOSES

Limit doses are maximal but reductions may be warranted in the dose level even in the absence of overt toxicity.

2.5.1 Oral Dosing

Regulatory agencies generally accept a maximum oral dosage rate of 1,000 mg/kg/day although this is not explicit in all guidelines. This is equivalent to 20,000 ppm (2%) in the diet for adult rats and 7,000ppm (0.7%) for adult mice (Farber, 1987). Some guidelines still quote 5% in diet, a value now usually confined to food additives. Pharmaceutical regulations for Europe and Japan allow a maximum dose of one hundred times the maximum human therapeutic dose. No limit is specified for nutritional agents.

2.5.2 Inhalation Dosing

It is generally accepted that the gaseous test material should not reduce oxygen concentrations of the inhaled air below 18%.

The maximum concentration for particulate exposures is 2 mg/litre (Whalan and Redden, 1994); this should be further reduced for poorly soluble and insoluble particulates which may saturate pulmonary clearance mechanisms and produce anomalous results (Hext, 1994).

2.5.3 Dermal Application Studies

There is a maximum quantity of any material that can be applied to animal skin so that it will remain in contact during the exposure period. The quantity varies with the species, the physical properties of the material and whether the application is occluded or not; it will usually be a thin layer. A maximum of about 10% of the skin surface area can be effectively exposed.

Skin irritation often sets the HDT in dermal carcinogenicity studies. The HDT for dermal studies was defined at an EPA workshop (US-EPA, 1989a,b) as the highest concentration which does not destroy the functional integrity of the epidermis, as determined by histology. Two other concentrations were recommended: the level which causes minimal irritation and the highest non-irritant level. Concentrations

which causè a marked inflammatory response, ulceration or necrosis were considered to be excessive since neoplasia may be secondary to these lesions. A set of photomicrographs of dermal lesions representing the spectrum of dermal irritation and dermal toxicity reactions were published to facilitate consistency in diagnostic terminology (US-EPA, 1990).

2.5.4 Other Routes

The properties, use and biological stability of the compound may occasionally require use of intramuscular or subcutaneous injection but this mainly applies to pharmaceutical agents. Dose is selected on practical grounds. Implantation is rarely used except for certain pharmaceuticals and medical materials. The general considerations for selection of the intravenous HDT are similar to those for the oral route. For repeated intravenous injections the volume should not normally exceed 5 ml/kg for isotonic solutions. For non-isotonic solutions this limit volume may need to be further reduced.

With subcutaneous or intracavity injections the concentration of soluble compounds used should minimise non-specific local tissue injury since this can lead to neoplastic reactions (Grasso and Golberg, 1966). For insoluble compounds the dose can be divided into a number of aliquots administered at intervals (typically weekly) in order to minimise discomfort to the test animals. For such compounds and for surgically implanted devices, a limit dose of 1 g/kg would normally apply unless the bulk of the preparation indicated a lower dose to be the maximum feasible.

2.6 REGULATORY PERSPECTIVES: CHANGING EXPECTATIONS OF THE "MTD" (HDT)

A number of factors make clear descriptions of "Not achieving" and "Exceeding" the "MTD" difficult. Requirements vary; in the case of the US-EPA separate guidelines exist for pesticides and industrial chemicals though their interpretation is consistent. International differences are substantial; e.g., the US-EPA carcinogenicity study guidelines and the EEC draft guidelines are not compatible in their definitions of acceptable effects at the MTD (Table 1, p.12). This is particularly apparent in the level of toxicity which has to be demonstrated: US agencies increasingly emphasise the need for a MaxTD, whereas the European regulators tend towards a MinTD to define the HDT.

A further complication is that the interpretation of the definition of "MTD" by each regulatory authority has changed; carcinogenicity studies which were acceptable 5 or 10 years ago may not now be acceptable, leading to the need for repeat studies.

TABLE 1: COMPARISON OF CRITERIA FOR SELECTION OF MTD (HDT) BY THE MAJOR INTERNATIONAL GUIDELINES

Criteria	Statement in Guideline or comments	OECD 451[1]	EEC 414[2]	US -FDA[3]	US -EPA[4]	Japan (MAFF)[5]
Basis of dose selection	Dose selection based on short-term testing	x	x	x	x	
	Doses tested may be selected on the basis of human exposure		x			
	Doses tested may be selected on the basis of metabolism and toxicokinetic data		x			
Body-weight decrement	Body-weight decrement not to exceed 10%	x				x
	Body-weight gain decrement of 10-15%in subchronic study				x	
	Slight depression in body-weight gain		x			
Signs, mortality	The highest dose should produce signs of minimal toxicity without altering the normal life span due to effects other than tumours	x	x		x	
	At the highest dose definite but minimal signs of toxicity are elicited without causing tissue necrosis or metabolic saturation	x	x			
	At the highest dose no severe changes in general conditions and no death due to toxicity in short-term toxicity test carried out for 1-3 months					x
	The highest dose is to be based on the type and gravity of a lesion and the likelihood of this lesion to influence the survivability of the high dose animals to the termination of the study			x		
	The MTD is that dose which, when given for the duration of the chronic study as the highest dose, will not shorten the treated animals' longevity from any toxic effects other than the induction of neoplasms.			x	x	
	The highest dose should not be selected too far below a life threatening level .				x	
Changes in other parameters	The MTD may also be established on changes in the following parameters: - haematological effects - histopathological changes - alterations in clinical chemistry - neurological symptoms - other clinical symptoms	x	x	x	x	x

[1] OECD (1981) [2] EEC (1991) [3]US-FDA (1993) [4] US-EPA (1984); Farber (1987) [5] J-MAFF (1985)

3. ISSUES FOR HUMAN RISK ASSESSMENT RESULTING FROM CURRENT APPROACHES TO "MTD"

3.1 HIGH DOSE PHENOMENA

Where significant lesions or disturbances of physiological status occur in test animals an increased incidence of tumours may be secondary to these events rather than reflecting any intrinsic carcinogenic activity of the test compound. Thus the relevance of such animal tumours for human carcinogenic risk at low exposure is questionable. Examples in which high doses produce such phenomena are given below.

3.1.1 Chronic Irritation and Cell Proliferation

While irritant substances are not necessarily carcinogenic, long-term administration of high, irritant doses can promote carcinogenesis in animals (Ames and Gold, 1990b). The mechanisms leading to cancer in chronically-irritated tissues are not fully understood. At cytotoxic doses, cells surrounding a spontaneously mutated cell may be killed, thereby removing signals inhibiting growth and permitting the mutated cell to proliferate. Reparative hyperplasia and mutagenic oxidants from phagocytic cells may increase the risk of mutation or genetic alteration (Gold *et al*, 1989). Prolonged and increased cell proliferation following cell injury may act as a promoter of carcinogenesis, for example chronic tissue regeneration subsequent to moderate proximal tubular damage caused by some petroleum hydrocarbons in the kidney is an important factor in renal tumour formation (Short *et al*, 1987). In skin, liver, fore-stomach and other organs, prolonged cell proliferation can also increase tumour formation while this is not seen at doses not causing proliferation (Craddock, 1976; Argyris, 1985; Rajewsky, 1985; Clayson *et al*, 1991a). Chronic irritation associated with bladder crystals, e.g. from prolonged ingestion of sodium saccharin has led to development of bladder tumours in male rats (Ellwein and Cohen, 1990; Cohen *et al*, 1991). The presence of calculi in the urinary bladder leads to tumours in rodents but not in man (Clayson, 1974; Clayson and Clegg, 1991). Calculi in the kidney can have the same effect. Calculi can be formed when uracil, melamine, calcium oxalate or diethylene glycol are administered, or they can arise secondary to metabolic changes (Cohen and Ellwein, 1992).

3.1.2 Saturation of Metabolism/Metabolite Accumulation

If the metabolic capacity of an animal is exceeded at high dose levels, the resulting high tissue concentrations of the test material or of metabolites may induce non-specific events, such as cellular linjury leading to impairment of protective cellular enzymes and a further increase in the local

concentrations of toxic chemicals (Carr and Kolbye, 1991). This is observed in methylene chloride-induced mouse tumours. The concentration of glutathione conjugate (the presumed active metabolite) increases as other metabolising systems become saturated, resulting in an increased number of tumours at high dose levels (Clayson and Clegg, 1991). In the liver, the excessive detoxification burdens at high doses can compromise defence mechanisms leading to liver tumours (Ciminera *et al*, 1988).

3.1.3 Mitogenesis

High doses of some substances can induce mitogenesis by interference with cell-cell communication. This effect can be supplementary to the replacement cell division following injury. As a dividing cell is much more at risk of mutation than a quiescent cell, mitogenesis increases mutagenesis (Ames and Gold, 1990 a,b).

3.1.4 Hormonal Imbalance

Disturbance of the hormonal balance in animals by test substances can also cause cancer. For example, drugs inhibiting thyroid hormone synthesis are goitrogens and thyroid carcinogens in experimental animals (Thomas *et al*, 1989; Thomas and Williams, 1991). The inhibition of thyroid hormone synthesis increases the secretion of thyroid-stimulating hormone and hence cellular proliferation. The frequency and type of tumours induced is influenced by the duration of administration, the dose level and the species tested (Thomas and Williams, 1991).

Some pharmacologically active compounds and non-genotoxic chemicals such as cimetidine (Leslie *et al*, 1981), metronidazole (Rustia and Shubik, 1979) and SDZ 200-110 (Roberts *et al*, 1989) are thought to cause Leydig cell neoplasia in rats via a hormonal mechanism. The hormonal changes are not seen in man at normal exposure levels.

3.2 CONSEQUENCES FOR RISK ASSESSMENT OF AN INAPPROPRIATE HIGH DOSE

The usual method of selection for the HDT assumes that any high dose toxicity can be extrapolated to low doses. This is not always the case. In extreme cases, excessive mortality at the HDT may render a study invalid. Where there is an adequate difference between the maximum likely human exposure and the NOEL and where the highest dose tested falls short of an "MTD" (however defined) there may be few problems in risk assessment. The acceptable margin of safety will differ with the class and use pattern of

a compound and must be considered on a case by case basis. While the toxicity found at high dosage levels generally indicates the risk at low dosage levels, the confidence that this is so is reduced when the dose tested exceeds human exposure by several orders of magnitude. There is little value in exposing animals to heroic doses, for example weekly administration to a rat of the amount of an agrochemical used to treat one hectare of a crop. Kociba (1987) suggested the inclusion of data on known or anticipated human exposure levels in dose selection. When doses are defined in this way, however, the bioassay designed for one particular use of a material may not be applicable to another.

4. OPTIONS FOR SELECTING HDT

4.1 RETAIN *STATUS QUO*

Despite their superficial similarity, the guidelines prepared by the OECD and the EEC at one hand and the US-EPA at the other (cf. Table 1) differ. This is an unsatisfactory situation.

4.2 REFINE *STATUS QUO*

The US Committee on Risk Assessment Methodology (CRAM, 1993) reported on the issues associated with MaxTD, particularly the definition currently used by the US-EPA. Two of the four options considered for setting bioassay dose levels were not meaningfully different from the *status quo* in that they maximise the dosage level with potential loss of specificity. The two other options were:

- use an HDT that is an arbitrary fraction of the estimated MaxTD;
- base the HDT on preliminary studies which determine the doses causing disturbances of physiological activity and the dependence of metabolism on dose.

The first retains the MaxTD as the HDT, when human exposures are 1/10 - 1/100 of this, or when there is a high probability that the test compound is a direct-acting carcinogen. For compounds that do not meet these criteria, the HDT would be a fraction of the estimated MaxTD, e.g. 1/2 or 1/3. This would focus attention and resources on those compounds of most concern, i.e., potential carcinogens and genotoxins, compounds structurally similar to hazardous substances and compounds with high human exposure.

The second option requires a greater initial commitment of resources. Based on investigations of toxic mechanisms and dose-response relationships, a bioassay would be designed which would have maximum value in assessing the human hazards. While many agree on the type of information useful in characterising toxicological responses, its application in setting the HDT is debated. Implementation of this is difficult with completely new chemical entities as development of knowledge of the toxic mechanism is essential.

A further possibility within this option would be to retain the overall concept of MaxTD but to redefine the criteria used for dose selection to minimise anomalies secondary to frankly toxic doses. In practical terms the HDT would be closer to the OECD MinTD rather than the current MaxTD. This would increase the

specificity of the bioassay by reducing the incidence of those tumours occurring secondarily to other toxic events.

4.3 TOXICOKINETICS

The increasing availability of toxicokinetic data allows them to be used more in dose setting. Where metabolism is saturated at a particular dose level this should be taken into account when setting the HDT.

Ideally human metabolic data should be derived, as is currently the case for pharmaceuticals, to allow comparison of the dose response curves of the test species and man and to avoid investigating a species which produces a metabolite not produced in man. Nevertheless, toxicokinetic data from animal experiments can be useful for dose setting even in the absence of human data.

4.4 AN INTEGRATED APPROACH

All available toxicity and toxicokinetic data should be used for the definition of HDT which should not exceed some arbitrary (eg 100 or 1,000 X) multiple of the probable human exposure. Kinetic parameters such as plasma concentrations, AUC values (area under the concentration-time curve), saturation of metabolism, elimination/excretion rates, disturbance of endocrine homeostasis, etc. would be important in selecting the route and frequency of administration. Body-weight, histopathological, toxicological and pharmacological profiles and the characteristics of the use of the compound should be taken into consideration in defining the HDT and intermediate levels.

4.4.1 Industrial Chemicals

The toxicokinetic information above can be derived from currently performed sub-chronic studies which include the toxicokinetic tests specified in Annex VIII of the EEC Dangerous Substances Directive (EEC,1988) and laborated by ECETOC (1992). Limited data on the metabolic route can be derived from *in vitro* systems but some estimate of probable exposure is also required. It is unlikely that human data would be available for such compounds but this could be derived from a model similar to the one that may be proposed for risk assessment under the 7th Amendment to the EEC Dangerous Substances Directive (EEC, 1992). In practice, selection of doses would be subject also to the establishment of a maximum feasible dose which should not exceed the "MTD" or limit dose. Selection of additional low doses (perhaps more than two) and appropriate controls would include consideration of probable dose-response relationships.

A disadvantage of this approach is that human exposure estimates used to define the HDT may change with different patterns of use. Direct comparison with close structural analogues may not be valid if the use patterns differ significantly.

4.4.2 Pharmaceuticals

Recent guidelines ensure an approach similar to that above for the selection of the HDT. Human exposure data are obtained during preliminary drug evaluation and this allows selection of the high dose on the basis of rodent/human AUC (Area Under the blood concentration Curve), ratios and saturation of absorption.

4.5 SEPARATE CRITERIA FOR CARCINOGENICITY AND CHRONIC TOXICITY EXPERIMENTS

The definition of HDT is currently applied identically to chronic toxicity and carcinogenicity studies but there is no reason why these two types of study should not be regarded separately. The studies are often combined to make most efficient use of laboratory animals and facilities. Toxic dose levels are desirable for proper definition of chronic toxicity but the lesions produced may cause a secondary carcinogenic response. Separation of the two experiments would allow the large number of parameters and organs investigated in carcinogenicity studies to be reduced without loss of validity and the chronic experiment to be extended in scope to include toxicokinetics and to define dose-response relationships.

If the studies are considered separately, the HDT can be defined separately in the following manner:

- for chronic toxicity experiments the HDT should elicit signs of toxicity; increased lethality should not be anticipated but may not invalidate the study; the HDT should not exceed a limit dose and when toxicokinetic investigations indicate saturation of absorption this will define the HDT;

- for carcinogenicity experiments the HDT should be below but close to the threshold where frank toxicity is seen in subchronic (or chronic) experiments; toxicokinetic investigations should indicate that metabolic overload has not been reached since in many cases this will be a dose inducing functional changes and/or adaptive responses.

Separation of carcinogenicity and chronic studies will prevent the induction of many tumours not directly related to the test substance, although increased cell necrosis, mitogenesis, long-term hormonal disturbances, sustained enzyme induction and peroxisome proliferation etc. might still induce tumours in some cases.

Restriction of a chronic toxicity study to at least 6-months (and not more than 12 months) could reduce the number of animals per treatment from the current 20, since more would survive to term. A group size of 15 would be adequate but a second high dose level could be added to chronic toxicity studies corresponding to the HDT for carcinogenicity. This modification would allow a more precise definition of the dose-response relationship for chronic toxicity and indicate the relationship of the HDT in the carcinogenicity study to that producing overt toxicity. Aside from a slightly reduced group size (15 versus 20) this concept does not deviate significantly from OECD Guideline 453 or from the design recommended by J-MAFF (1985).

TABLE 2: ANIMAL NUMBERS IN CURRENT AND PROPOSED DESIGN FOR A COMBINED CHRONIC TOXICITY/CARCINOGENICITY STUDY

	Current Design		Proposed Design	
Dose Group	Carcinogenicity	Chronic Toxicity	Carcinogenicity	Chronic Toxicity
Control	50/sex	20/sex	50/sex	15/sex
Low dose	50/sex	20/sex	50/sex	15/sex
Mid dose	50/sex	20/sex	50/sex	15/sex
High dose (Carcinogenicity)	50/sex	20/sex	50/sex	15/sex
High dose (Chronic toxicity)				15/sex
Totals	280/sex		275/sex	

4.6 ADDITIONAL DOSE LEVELS

In addition to the control and HDT groups, current recommendations include a low-dose group of one-fourth to one-tenth the HDT and an intermediate dose group. In practice this design has limited application as many biologically-active test substances show significant toxicity at one-tenth HDT and the lower dose is expected to be a no-effect level. For non-pharmaceutical substances when the HDT is set several orders of magnitude above human exposure levels the low dose is often set at 100 to 1,000 times projected human exposure to reduce the uncertainty of extrapolations to the human situation. The

intermediate dose is selected to define better the dose-response relationships. Considerations of the linearity of the toxicokinetics, the potential for metabolic saturation and altered metabolism, the need for a NOEL to cover exposure, occurrence of physiological changes and the need for mechanistic information help select the intermediate dose. Where compounds induce changes of no toxicological importance (e.g. liver enzyme induction leading to hypertrophy) there may be a need to define a dose where this change is evident but where no other changes are manifest (i.e. a no observed **adverse** effect level - NOAEL).

In some cases additional dose groups can significantly improve study design. A group dosed at half to one-third of HDT can ensure a satisfactory study where the HDT proves too high and doses at points of probable biological significance (e.g. metabolic break points or levels at which only pharmacological effects are expected) may give useful information. Where there is a very shallow dose-response curve additional dose groups may be added to avoid excessive separation of dose levels used (i.e. by more than an order of magnitude).

While these designs may help to resolve interpretive difficulties, which are frequently predictable in advance, their wider adoption has some disadvantages. Extra groups will increase animal usage, without any guarantee of a proportional increase in the value of the information derived. Resource requirements and bioassay costs would be substantially increased and evaluation would take longer.

4.7 LIMIT DOSES

The rationale and working definitions of limit doses are detailed in Section 2.6. A limit dose of 1% in the diet for rats and mice has been suggested by Apostolou and Helton (1993) and a maximum exposure rate of 500 mg/kg/day proposed by Monro and Davis (1993).

Pharmaceutical regulations for Europe and Japan allow a maximum dose of 100 times the maximum therapeutic dose. To allow for pharmacokinetic differences between species, replacement of this limit by a minimum factor of 25 times the human AUC where there is a linear relationship between AUC and dose has been proposed (ICH, 1992, 1993). This approach is not yet available for other products because human studies are not performed in their early development.

For industrial and agro-chemicals the HDT could be defined as multiples of human exposure levels but while maximum human exposures can be estimated for many of them, the assumptions necessary may be considerable. International harmonisation on methods for exposure estimation and the multiple of human exposure used to define the HDT for such chemicals is desirable.

5. APPRAISAL

5.1 ADVANTAGES AND DISADVANTAGES OF "MTD" CONCEPTS

The reasons for and against using an "MTD" (whether MaxTD or MinTD) in carcinogenicity studies are summarised below. Oral administration is the most common mode of administration in long-term studies but arguments are similar for other exposure routes.

The major advantage is that animal numbers employed in conventional bioassays are small so an exaggerated dosing regime maximises their sensitivity (Kociba, 1987; Howell *et al*, 1988; McConnell, 1989; Carr and Kolbye, 1991; Faccini *et al*, 1992). The challenge is sufficient to identify oncogenic hazards (Farber, 1987) and the chance that a weaker carcinogen would be undetected is minimised (Carr and Kolbye, 1991). No retesting is necessary should higher and potentially toxic human exposures occur (Carr and Kolbye, 1991; Clayson *et al*, 1991b). Both MaxTD and MinTD definitions would achieve these objectives with a posible slight loss of sensitivity in the case of agents where carcinogenic potential is secondary to toxicity. Assays conducted at the MaxTD provide a warning that where human beings develop toxic lesions from exposure to a non-genotoxic agent, they may be at risk of developing cancer (Clayson *et al*, 1991b; Faccini *et al*, 1992). In the absence of information on plasma concentrations evidence of toxicity is frequently used to confirm that absorption has occurred and hence that the study is valid (Faccini *et al*, 1992). These latter advantages would not always be seen with studies conducted at the MinTD.

The major disadvantage of administering chemicals at the MaxTD is that the phenomena detailed in section 3.1 may occur resulting in difficulties in extrapolation of results to man (Ciminera *et al*, 1988; Gold *et al*, 1989; Ames and Gold, 1990 a,b; Carr and Kolbye, 1991; Cohen and Ellwein, 1992; Faccini *et al*, 1992). High doses of low toxicity compounds can overload metabolic pathways (Kociba, 1987; Kodell, 1988) or modify metabolic processes (Faccini *et al*, 1992), leading to abnormally high tissue concentrations of test materials or generation of metabolites not found at normal exposure. Such experiments do not fulfil the primary purpose of the experiment and may violate animal use ethics; chemicals found to be carcinogenic at these high doses may represent no hazard under conditions of normal exposure (McConnell, 1989; Faccini *et al*, 1992). The application of the MinTD concept in dose selection will reduce the incidence of these problems.

That the "MTD" is inconsistently defined by regulatory agencies (McConnell, 1989; NACA, 1991) exacerbates the problems of interpretation of carcinogenic activity seen only at doses exceeding the "MTD", so that data are controversial and subject to criticism and legal challenges (Carr and Kolbye, 1991).

5.2 SELECTION OF HIGHEST DOSE TESTED

5.2.1 General Principles

Provided preliminary studies are expertly interpreted, the best criterion for HDT in cancer bioassays would be one which closely approaches a toxic dose (or the limit dose) so that assessment is rigorous and comparable data are available to evaluate structure-function relationships. The major question is how this dose should be defined.

If based on results of short-term, sub-chronic and toxicokinetic studies and estimates of human exposure (the integrated approach), the HDT can provide data more readily extrapolated to man. Much of the required information is available for pharmaceutical compounds but their determination for agricultural and industrial chemicals would be impractical, although some animal toxicokinetic data which would help in dose setting could be obtained at minimal cost. When one or more of these elements is not available or subject to uncertainty or where the HDT causes excessive toxicity, the validity of the risk assessment may be considerably diminished.

The argument that testing at a dose even marginally below the MaxTD may fail to detect cancer which occurs secondary to other toxicity has validity only if the bioassay is considered a qualitative screen for carcinogens. In quantitative risk assessment it must be recognised that such effects either are subject to a threshold or require a completely different approach to that used with genotoxic carcinogens. Setting the HDT at a level producing no significant physiological disturbance, rather than just below a life-threatening level, would reduce the incidence of spurious classification. Consideration of dose-responses for genotoxic and non-genotoxic carcinogens suggests that for maximal sensitivity the HDT should not be far below the level causing frank toxicity.

The use of the OECD/EEC MinTD for carcinogenicity tests and the US MaxTD for chronic toxicity testing may be an acceptable compromise. The chronic test would then identify responses indicative of non-genotoxic carcinogenicity and aid the definition of a no-effect level for them and the MinTD would free the carcinogenicity assessment from the interpretive complications.

This does not negate the need to define a limit dose. All test systems have a dosage level beyond which reliable results cannot be obtained; failure may be catastrophic, and hence self-limiting, or the animals may survive even though physiologically compromised. Examples are reduction of food intake by a chemical to the point where vitamin deficiency occurs, or a chelating agent inducing mineral deficiency. Such properties present few interpretive difficulties if the mechanism is understood but could mislead with a new chemical with a weak effect after prolonged high-dose administration. The use of a limit dose minimises such spurious results.

The use of human exposure levels in setting limit doses has been commented upon. The argument that dose levels should be maximised to enhance identification of effects is countered by the view that effects seen only at high dose levels cannot be extrapolated to dose levels orders of magnitude lower and that excessively high doses reduce the specificity of a test.

Most regulatory authorities accept an HDT of 1,000 mg/kg/day. With the integrated approach this is considered acceptable since by definition it would be confined to compounds of low toxicity with significant potential for human exposure.

5.2.2 Application to Various Routes of Exposure

Oral Studies

As the oral route of exposure is the choice for most investigations, discussion of dose-setting procedures tends to focus on this. The major problem is to ensure a dose which adequately tests for carcinogenicity without inducing excessive responses. The separation of HDT for chronic toxicity from that for carcinogenicity should simplify interpretive problems.

The HDT will usually be selected from the results of a sub-chronic (90 day) study, the doses for which are selected to allow adequate expression of intrinsic toxicity, i.e. the high dose should not be set too low and the intermediate dose(s) should define a dose-response relationship. With compounds of low toxicity it is not necessary to exceed the chronic toxicity limit-dose as young, rapidly-growing animals, with maximum susceptibility to toxic effects, are used.

The same conditions should apply to the selection of the HDT for the carcinogen bioassay on a second species (usually the mouse) on which chronic toxicity may not be evaluated simultaneously. Preliminary studies should show that the HDT is close to the toxic dose and that the dose-response curve is similar to that in the primary species (usually the rat). Marked differences between the species may indicate different metabolism of the test compound, in which case the relevance of each species to man should be evaluated before the carcinogenicity study is conducted.

Other Routes

The same principles apply to other routes; limit doses may be based on route-specific irritancy or physical chemistry.

5.2.3 Adaptive and Pharmacological Effects

Some compounds induce metabolic enzymes, particularly in the liver, and lead to an increase in organ weight with little or no histological change. Adaptive response to an increased metabolic load is reversible when the compound is withdrawn and should be ignored when selecting HDT unless the adaptive change becomes excessive, (e.g. hypertrophy of a magnitude which would not readily reverse or increased cell turnover): these factors are exemplified in Section 6.

Pharmacological effects should be treated similarly but compounds with direct or indirect hormonal activity should be considered with particular care as they may cause tumours by non-genotoxic mechanisms (see Section 3.1).

Effects specific to test species may also be disregarded when selecting the HDT unless they shorten the lifespan significantly. Tumours in organs so affected should be ignored, potentially reducing the sensitivity of the assay. The use of an alternative model should be considered in these circumstances.

6. PRACTICAL CONSIDERATIONS AND RECOMMENDATIONS

6.1 PRACTICAL GUIDELINES FOR DOSE SELECTION FOR LOW TOXICITY COMPOUNDS

The HDT should not exceed limits laid down in regulations or reasonable practical or feasible maxima (c.f. Section 2.6). It is recommended that, additionally, the limit HDT should be based on multiples of human exposure where these are predictable. For pharmaceuticals an HDT of 25 or more times the AUC human equivalent and for other chemicals a dose of 10,000 times the likely human exposure is considered adequate for risk assessment; this allows for a two order of magnitude span of dose levels in the study coupled with a 100-fold safety factor over the NOEL.

6.2 PRACTICAL GUIDELINES FOR DOSE SELECTION BELOW THE LIMIT DOSE

Different criteria apply to chronic toxicity and carcinogenicity studies. In the former, dose levels may be selected which cause frank toxicity, so that the HDT for a chronic toxicity study may be excessive if encountered in an oncogenicity study, and tumour information obtained at this dose level may have to be disregarded in risk assessment. Unexpected levels of toxicity in the chronic study may not invalidate interpretation of non-tumour effects.

Dose setting should be based on results of sub-acute and/or sub-chronic studies or of studies of longer duration, where available. Criteria (Section 6.2-6.7; Table 3, p.28) are exemplified for the target levels of toxicity which should be aimed for in chronic toxicity and carcinogenicity studies. The criteria are based on experience with the laboratory rat. In principle they can be applied to other species although expert judgement on the applicability of the values cited is required. These criteria should be used as targets in dose selection; whether or not the criteria are achieved will be known only on completion of the study when the magnitude of the observed effects can be compared with the predictions. Nevertheless, if the doses selected are based on existing data and would reasonably be expected to give the levels of effect indicated in the table, failure to reach the predicted levels of toxicity should not invalidate the study. It may be possible to ignore pharmacological responses and adaptations to metabolic overload unless they are persistent or become excessive (see Section 5.2.3).

TABLE 3: EXAMPLES OF CRITERIA FOR DEFINING HDT IN CARCINOGENICITY AND CHRONIC TOXICITY STUDIES

Indicative Criteria for Acceptability

Parameter	Carcinogenicity Study	Chronic Study
Mortality	<5%	<5%
Clinical Signs	Minimal or slight behavioural changes	Moderate behavioural changes
Reduction in Body-weight Gain: diverging converging or parallel	 5% 10%	 5-10% 10-15%
Haematology and Clinical Pathology: Haemoglobin reduction Leukocyte count Platelets Clotting Time (prothrombin time) Plasma gamma GT (rodents) Transaminases Cholesterol/triglycerides Glucose	 ≤ 2g/dl Hb 60-200% of control 50-150% of control x 2 ≤ 5 i.u. x 2 x 2 60-150% of control	 ≤ 3g/dl Hb 40-400% of control 30-200% of control x 2-3 ≤ 25 i.u. x 4 x 3 40-200% of control
Physiological Function:	see text	see text
Organ Weight Increase: Liver (mice) Liver (rats) Liver (dogs) Spleen and hormonally controlled organs	 ≤ 50% ≤ 25% – 50-300% of control	 ≤ 100% ≤ 50% ≤ 30% 0-300% of control
Histopathology:	see text	see text
Cytotoxicity: Cytogenicity; Mitotic index Apoptosis Single cell necrosis Focal or multifocal necrosis Other effects; Chronic inflammation Atrophy Haemorrhage	 Slight increase Minimal/slight increase in most animals Minimal/slight increase in at least 10% of animals None None Slight increase No increase in observed incidence	 Moderate increase in all animals Slight/moderate increase in most animals Moderate effects in 20% of animals or slight effects in all animals Minimal or slight in 10% of animals Less than moderate increase Moderate to severe increase Slight increase in observed incidence
Metabolic Overload	see text	see text

Note: Ranges of effect given, which include 100%, indicate that the parameters could have compound related decreases or increases which would be considered toxicologically significant.

Some of the effects listed are sufficient to establish the validity of the HDT (definitive parameters). Others provide supportive evidence but may also provide adequate evidence of an effect if the next highest dose proves excessive. It is emphasised that the figures and descriptions in each parameter should be considered as indicative rather than absolute. Dose selection for males and females should be the same unless there is strong evidence for a sex difference.

6.2.1 Mortality

Significantly-raised mortality is indicative of excessive dosage. There should be no (or nearly no) increase in mortality in the first year in either chronic toxicity or carcinogenicity studies. Subsequent mortality in carcinogenicity studies should be only that which is tumour-related. Increased mortality is a definitive parameter in a carcinogenicity study.

6.2.2 Clinical Signs

These may limit the HDT. Some clinical signs, (e.g. paralysis, self-mutilation and violent aggression) are unacceptable on ethical grounds. Clinical signs which indicate a potential for reduced life span should be taken into account. Ethically based clinical signs are a definitive parameter for setting an HDT.

6.2.3 Body-weight Gain

Changes in body-weight gain are most frequently used as a criterion for selecting the HDT. Transient reduction (seen only in the first few weeks of a study) would not normally be the sole determinant of HDT but a trend which continued throughout most of a 90 day study is of importance. If the mean body-weight of a treated group continues to diverge from that of the controls, a difference at 90 days of 5% (for carcinogenicity) or 5-10% (for chronic toxicity) is considered of significance in dose setting. If the growth curves are parallel or convergent, a criterion of target growth curve differences of 10% and 10-15% respectively at 90 days should be adopted. Body-weight change is a definitive parameter.

6.2.4 Haematology and Clinical Pathology

Haematological and clinical pathological changes may determine the HDT when sub-chronic studies show a continuing effect which indicates a significant physiological abnormality or reduced viability. Examples of criteria which might reasonably be used are given in Table 3. Such effects may need support when setting an HDT.

6.2.5 Physiological Function

Changes in the physiological function are often detected by observation or identified through clinical pathological changes. Significant changes in physiological function can alter tumour incidence, e.g. severely altered hormone levels may result in atrophy or over stimulation of responsive tissues. Where such changes are unlikely to occur in man, the animal model should be avoided or the results disregarded. Moderate to extreme effects are definitive, lesser effects are seldom definitive in setting the HDT.

6.2.6 Organ Weight Increase

Organ weight changes reflect metabolic, physiological or toxic lesions in tissues. Histological changes are generally the best indices for determination of the dose-response effect but metabolic effects, (e.g. liver hypertrophy) and physiological effects, (e.g. hormone-related thyroid hypertrophy) may be shown up by changes in organ weight.

Major changes in organ weight are indicative of major disturbances in cellular metabolism or cell turnover, or of pathological injury. Liver weights may be increased by various adaptive responses, notably by enzyme induction. Although generally disregarded in dose setting, when present at extreme levels over a prolonged period in a carcinogenicity study it may influence the findings. The level at which such changes may be considered extreme is indicated in Table 3. For mice in particular, the tabulated levels of increase may, if prolonged, be sufficient to induce significant tumour formation in the liver by non-genotoxic mechanisms. Organ weight changes are regarded as a definitive parameter.

6.2.7 Histopathology

The tissues affected, the type of change and the severity must be evaluated when determining the significance of an observation for dose-setting. The wide range of possible histopathological abnormalities precludes listing all; expert pathological evaluation is an essential component of dose-selection. Histological evaluation frequently adds perspective to clinical pathological and physiological function data. Histopathological changes are often definitive in determining the HDT.

6.2.8 Cytotoxicity

Cytotoxic effects in a tissue, as evidenced by changes in mitotic index, apoptosis or necrosis, may increase or decrease the rate of tumour expression and confound interpretation of carcinogenicity

studies. Special studies on the rate of cell turnover may be used to confirm and quantify the histological observations.

6.2.9 Metabolic Overload (Toxicokinetics)

If toxicokinetic measurements indicate saturation of absorption, the dose at which this occurs defines the HDT. Finding a metabolic overload also affects dose selection, as a changed metabolic route or toxicokinetic profile is not appropriate in carcinogenicity studies. It may, however, be necessary to test at high dose levels if this induces a route of metabolism known to operate in man.

6.2.10 Exceptions

Careful judgement must be applied in all cases and specific circumstances may warrant the adoption of different criteria to those listed in Table 3.

7. RECOMMENDATIONS FOR REGULATORY CHANGES

It is recommended that the regulatory authorities adapt their guidelines for chronic toxicity and carcinogenicity studies so that:

- the concept of limit doses should be continued but their definition should include multiples of human exposure as one criterion;
- there should be separate criteria for the selection of the HDT in carcinogenicity and chronic toxicity studies;
- the current US-EPA definition of MTD (MaxTD in this report) - (Farber, 1987) should be confined to chronic toxicity studies;
- definition of HDT for carcinogenicity studies should conform more closely to the "minimally toxic" criteria currently used by OECD and EEC (MinTD in this report);
- clear criteria for defining excessive toxicity in carcinogenicity studies should be adopted; dose levels producing such toxicity should not be used for hazard or risk assessment: in general, a chronic toxicity HDT will be excessive for carcinogenicity studies;
- chronic toxicity studies should be of at least 6-months duration, but no more than 12 months since little additional information is obtained beyond this time;
- the chronic toxicity study could incorporate the dose levels employed in a carcinogenicity study but an additional higher dose level may be required.

APPENDIX A. DEFINITIONS OF MAXIMUM TOLERATED DOSE ("MTD").

The "MTD" has been defined in the following manner.

OECD

The highest dose level should be sufficiently high to elicit signs of minimal toxicity without substantially altering the normal life span due to effects other than tumours. Signs of toxicity are those that may be indicated by alterations in serum enzyme levels or slight depression of body-weight gain (less than 10%)" (OECD, 1981).

Sontag *et al*

"The MTD is defined as the highest dose of the test agent during the chronic study that can be predicted not to alter the animals' normal longevity from effects other than carcinogenicity.. (and) which, in the subchronic study, causes no more than 10% weight decrement, as compared to the appropriate control groups, and does not produce mortality, clinical signs of toxicity, or pathologic lesions (other than those that may be related to a neoplastic response) that would be predicted (in the chronic study) to shorten an animal's natural life span" (Sontag *et al*, 1976).

Food Safety Council

"...The high dose in chronic testing which should be defined as one which in a subchronic study:

- induces no overt toxicity, i.e. appreciable death of cells or organ dysfunction as determined by appropriate clinical pathological, pathological or biological methods;
- induces no toxic manifestations which are predicted to shorten the lifespan of animals except as a result of neoplastic development;
- in two generation studies, is not detrimental to conception rates, fetal or neonatal survival, or postnatal development;
- does not retard weight gain during the subchronic test by greater than 10% as compared to control animals;

- takes into consideration metabolic and pharmacokinetic data, and if dose-dependent qualitative or quantitative differences occur, at least one dose level should be set above the metabolic shift (provided the level(s) does not exceed the criteria list above..." (Food Safety Council, 1978).

Ruckelshaus

"Positive studies at levels above the MTD should be carefully reviewed to ensure that the responses are not due to factors which do not operate at exposure levels below the MTD. Evidence indicating that high dose testing procedures produces tumour responses by indirect mechanisms that may be dealt with on an individual basis" (Ruckelshaus, 1984).

Paynter

"...The largest administered dose should be one which produces signs of minimal toxicity that does not compromise biological interpretability of the observed responses. For example, the upper dose should not: (a) alter survival in a significant manner due to effects other than tumour production; (b) cause body-weight decrement from the concurrent control values of greater than 10-12%; (c) exceed 5% of the total diet: (d) produce toxic, pharmacologic, or physiologic effects that will shorten duration of the study or otherwise vitiate the study results" (Paynter, 1985).

IARC

"The high dose in the chronic study is one which is expected on the basis of an adequate subchronic study to produce some toxicity when administered for the duration of the test period. It should not, however, induce (a) overt toxicity, i.e. appreciable death of cells or organic dysfunction, as determined by appropriate methods; (b) toxic manifestations which are predicted materially to reduce the lifespan of the animals except as a result of neoplastic development; or (c) 10% or greater retardation of body-weight gain as compared with control animals" (IARC, 1980).

US - Office of Science and Technology Policy

"The highest dose level currently recommended is that just high enough to elicit signs of minimal toxicity without significantly altering the normal lifespan due to effects other than carcinogenicity...This maximum tolerated dose (MTD) is ordinarily determined in a 90-day study; and was originally based on a weight gain decrement, i.e. the highest dose that will produce a slight depression in body-weight (approximately 10-12%) if administered over a lifetime. NTP has more recently suggested refinement of the MTD selection on the basis of a broader range of biological information. Other signs of toxicity that may be used to establish the MTD include primarily gross and microscopic pathology, but alterations in serum

enzyme levels, haematological effects or other physiological, biochemical or pharmacological indices of abnormality may be useful.... High doses themselves may produce physiologic conditions which can qualitatively effect the induction of malignant tumours. Normal physiology, homeostasis and detoxification or repair mechanisms may be overwhelmed and cancer, which otherwise may not have occurred, is induced or promoted. If qualitatively different distribution, detoxification or elimination of test chemical is produced at the highest dose, a toxic response at this dose may not be indicative of effects at low dose levels. The cancers which arise from high doses of corticosteroids, oestrogens, certain sulphonamide compounds and in some instances of bladder implantation may result from such "secondary" effects. ... There should be reasonable scientific certainty that the dose used meets the objectives of maximally enhancing the sensitivity of the test without introducing qualitative distortions in the results" (Office of Science and Technology Policy, 1984).

US Government Interagency Regulatory Liaison Group

"...The estimated maximum tolerated dose (EMTD) is defined as the highest dose that can be administered to the test animals for their lifetime and that is estimated not to produce (a) clinical signs of toxicity or pathologic lesions other than those related to a neoplastic response, but which may interfere with the neoplastic response; (b) alteration of the normal longevity of the animals from toxic effects other than carcinogenesis; and (c) more than relatively small percent inhibition of normal weight gain (not exceed 10%)" (Interagency Regulatory Liaison Group, 1979).

National Cancer Advisory Board

"...The National Cancer Advisory Board (has) cautioned against the use of a dose so high that it produced ..."unphysiologic conditions (which) may in themselves enhance tumour formation"" (National Cancer Advisory Board, 1977).

International Life Sciences Institute

"The high dose should not exceed that dose at which toxic effects are noted in subchronic studies and it should elicit, if possible discernable signs of toxicity. ....the doses selected for the chronic study should not cause toxicity which might have pronounced secondary effects on parameters such as growth rate, immune status or survivability. ...in carcinogenicity testing, it is paramount to distinguish when possible between those substances that induce cancer through direct interaction with genetic material and those that induce tumours by overwhelming normal physiological functions" (ILSI, 1984).

UK Department of Health

"..for the best chance of detecting (carcinogenic) activity, a chemical should be administered at a dose level just within the toxic range (e.g. a level which causes 10% reduction in body-weight gain and/or minimal target organ toxicity" (UK-DOH, 1979).

World Health Organisation

"(The highest dose) should produce some slight evidence of toxicity, but should be compatible with normal physiological function" (WHO, 1978).

EEC Scientific Committe for Food

"The highest dose should be one which in subchronic tests induces no overt toxicity or toxic manifestations, and is predicted not to shorten the lifespan of animals except as a result of neoplastic development. It should not retard weight gain by more than 10% as compared to the control" (EEC, 1980).

APPENDIX B. COMPARISON OF REGULATORY GUIDELINES AND PRACTICES

B.1 CARCINOGENICITY STUDIES: INDUSTRIAL CHEMICALS AND AGROCHEMICALS

(OECD,EEC, UK-HSC, US-EPA (TSCA), US-EPA (FIFRA), J-MAFF)

STUDY DESIGN

Animal species:

For a compound of unknown activity, assays with two animal species are recommended, rats and mice being preferred. Syrian hamsters are mentioned as being particularly suitable for studies on respiratory tract carcinogenesis.

Group size:

At least 50 male and 50 female rodents are required for each dose level and for the control(s). If interim killing is planned, the initial number should be increased by the number of animals scheduled for the interim sacrifice(s).

Determination of the HDT:

The principle parameters which determine the HDT tested are based on body-weight effects, histopathology, haematologic effects, clinical chemistry, urinalysis and organ-weight changes (c.f. Table 2) based on guidelines and their interpretation as compiled by OECD (1981),EEC (1985), US-EPA (1983) and J-MAFF (1985). Although there are similarities with the testing of pharmaceutical agents it should also be noted that with current practices toxicokinetic data are rarely available for industrial and agricultural chemicals at the time of dose selection.

Dose levels:

The selection of the dose levels should be based on data from previous toxicity studies, preferably subchronic studies (EEC, OECD). Three dose levels, in addition to a control, are recommended so that information on the dose-response relationship can be obtained.

Maximum Dose:

The high dose should be sufficiently high to elicit signs of minimal toxicity (e.g. a depression of body-weight gain of less than 10%) without substantial alteration in the normal life-span due to effects other than tumours (except US-EPA, US-EPA(TSCA) and US-EPA(FIFRA)).

A dose of 1 g/kg body-weight/day should provide an adequate upper limit for the testing of most pesticides and industrial chemicals. For dietary administration the equivalent dose is deemed to be 2% in the diets of rats and 0.7% for mice.

For diet mixtures, the highest concentration should not exceed 5%, with the exception of nutrients testing (OECD).

Lower Doses:

The lowest dose should not interfere with the normal growth, development and longevity of the animals and it must not otherwise give any indication of toxicity. In general, the low dose should not be less than 10% of the high dose (OECD,EEC, UK-HSC, US-EPA(TSCA)).

At least two lower dose levels should be used. The lowest dose should not produce any effect and will normally exceed the maximum permitted human exposure level if this is known (J-MAFF, US-EPA (FIFRA)).

The intermediate dose should be in the mid-range between the high and the low dose. OECD and US-EPA(TSCA) mention the use of toxicokinetic properties of the chemical, if known, in setting the intermediate dose. Intermediate dose(s) may be placed at the geometric mean or at level(s) to define no-effect levels for adaptive or pharmacological change. In some instances the intermediate dose may be at or close to the point of saturation of the primary metabolic route.

Duration of treatment:

This depends on the normal life-span of the strain of test animal. In general, for mice the test is terminated at 18 months, and for rats at 24 months. With certain strains of mice or rats of greater longevity or low spontaneous-tumour rate, the termination may be postponed to 24 or 30 months, respectively.

Alternatively, termination of the study is acceptable when the number of survivors in the lowest dose or control group falls to 25%. When there is an apparent sex difference in response, the results from each sex should be considered separately.

"For a negative test to be acceptable" (OECD,EEC, UK-HSC), not more than 10% of any group may be lost due to autolysis, cannibalism or management reasons, and survival of all groups should be at least 50% at 18 and 24 months in the case of mice and rats, respectively.

STUDY OBSERVATIONS

Clinical data:

The animals are observed daily by physical examination. Special attention must be paid to tumour development. The time of onset, location, dimensions, appearance and progression of each grossly visible or palpable tumour should be recorded. Detailed clinical examination of sick animals is necessary to provide a diagnosis.

Haematology:

At 12 and 18 months and on killing, a blood smear is obtained from 10 animals/sex/group (US-EPA(FIFRA) or from all the animals (all other Guidelines). A differential blood cell count is performed on samples of the animals in the highest dose group and the controls. If necessary, it is also performed on samples of the next lower dose group and on sick animals.

Post-mortem examinations:

Complete gross pathology should be carried out on all animals. All visible tumours, and lesions suspected of being tumours, should be preserved. Organs and tissues of all animals should be preserved for microscopic examination. Organ weight determinations are requested by US-EPA (FIFRA) and J-MAFF.

Histopathology should be performed :

- on all grossly-visible tumours, or lesions suspected of being tumours, in all groups;
- on all preserved organs and tissues of the animals that died or were killed during the study;

- on all preserved organs and tissues of the highest-dose group and controls;

- on the organs or tissues of all animals in the study in which significant differences in hyperplastic, pre-neoplastic or neoplastic lesions are observed between the highest dose and control groups.

If excessive early death or other substantial alterations occur in the highest dose group, the group at the next lower dose level should be examined as described for the highest dose group.

B.2 CARCINOGENICITY STUDIES: PHARMACEUTICALS
(EEC, US-FDA, J-MHW)

STUDY DESIGN

Animal species:

Carcinogenicity studies with two rodent species are recommended. Rats and mice with a well established tumour profile are preferred. As an alternative, Syrian hamsters are mentioned.

Group size:

A minimum of 50 male and 50 female rodents are required for each dose level and for the control(s) (e.g. untreated/treated control group). 100 male and 100 female rodents for the control group are recommended by EEC guidelines.

Definition of the Highest Dose to be Tested (HDT):

The HDT in a carcinogenicity study is a dose producing only minimal signs of toxicity, and is set close to the threshold where frank toxicity is seen in subchronic or chronic studies; toxicokinetic investigations should indicate that metabolic overload has not been reached. It is the highest dose administered to animals for their lifetime that can be predicted not to influence survival or inducing life-threatening effects (eg the dose should elicit significant toxicity without substantially altering the normal life-span of the test-animal other than through tumour formation).

Dose Levels:

At least 3 dose levels are recommended.

Maximum Dose:

The highest dose should be the MaxTD (US-FDA), which should produce a minimum toxic effect (EEC) e.g. up to 10% reduction in body-weight gain or minimal toxicity in target organs. The highest dose may also be defined as 100 times the human therapeutic dose on a mg/kg basis (EEC, J-MHW). In feeding studies the dose should not exceed 5% in the diet. (J-MHW, 1990; EEC, 1983; ICH/EFPIA, 1993).

Lower Doses:

At least two lower doses should be employed. The lowest dose should not induce any toxic effect and be 2 to 3 times the maximum therapeutic dose or a pharmacologically active dose in animals (EEC). According to the Japanese guidelines it should be a no-toxic-effect dose and should be at least 10% of the highest dose.

The intermediate dose should be the geometric mean of the high and the low doses.

Duration of treatment:

Generally, for rats the study is terminated at 24 months with an extension to up to 30 months for long-lived strains; for mice or hamsters the study is terminated at 18 months, with an extension to up to 24 months. Alternatively, termination of the study is acceptable when the survival rate of the controls or low-dose group is 20-25%. Apparent differences in the mortality rate should be considered separately for each sex.

A prerequisite for the acceptance of a negative result is a survival rate of at least 50% of all groups at 18 and 24 months in the case of mice and rats respectively and not more than 10% of any group may be lost due to autolysis, cannibalism or management reasons.

STUDY OBSERVATIONS

Clinical data:

Mortality should be examined at least once daily for all animals, body-weight and food consumption should be recorded on a weekly basis for the first 3 months, thereafter once a month and palpation should be performed on a weekly basis throughout the study.

Haematology:

On killing, peripheral RBC and WBC should be counted, blood smears should be prepared (J-MHW). At 12 months, 18 months and prior to killing a blood smear is obtained from all animals. A differential blood count is performed on samples from the animals of the high dose group and the controls. If indicated, counts should also be performed on the next lower group(s) (EEC).

Post-Mortem Examination:

Gross necropsy should be performed on all animals. Organs and tissues of all animals and all visible tumours or lesions suspected of being tumours should be preserved.

Histopathology should be performed on all grossly-visible tumours or lesions suspected of being tumours in all groups, on the organs and tissues of all animals which died or were killed during the study. Further histopathology should be carried out on all animals of the control and high-dose group.

If there are differences in the incidence of neoplastic/preneoplastic lesions then the particular organ/tissue should be examined histopathologically in the other (mid- and low-) dose groups.

If survival of the high-dose group is substantially less than the control, or if there is evidence of induction of toxic or other effects in the high-dose group which might affect a neoplastic response, then the next-lower dose group should be examined fully.

B.3 CHRONIC TOXICITY STUDIES: INDUSTRIAL CHEMICALS AND AGROCHEMICALS (OECD, US-EPA, J-MAFF)

STUDY DESIGN

Animal species:

Two mammalian species are recommended, a rodent and a non-rodent (OECD, US-EPA, J-MAFF). The rat is the preferred rodent species and the dog is the preferred non-rodent species.

Group size:

At least 20 male and 20 female rodents and at least 4 male and 4 female non-rodents are required for each dose level and for the control(s). If interim killings are planned, the initial number should be increased by the number of animals scheduled for the interim killing(s).

Dose levels:

Three dose levels in addition to the concurrent control group are recommended so that information on the dose-response relationship as well as no-observed-toxic-effect level can be obtained (OECD, US-EPA, J-MAFF).

The highest dose level in rodents should elicit some signs of toxicity without causing excessive lethality. For rodents, the incidence of fatalities in low- and intermediate-dose groups and in the controls should be low to enable a meaningful evaluation of the results. For non-rodents, there should be no fatalities.

The lowest dose level should not produce any evidence of toxicity. Where there is a usable estimation of human exposure, the lowest dose level should exceed this. The intermediate-dose level(s) should produce minimal observable toxic effects. If more than one intermediate dose is used, the dose levels should be spaced to produce a gradation of toxic effects (US-EPA).

For diet mixtures, the highest concentration should not exceed 5%, with the exception of nutrients (OECD, US-EPA, J-MAFF).

Duration of treatment:

The duration of the exposure period in rodents for chemicals intended for a non-food use should usually be at least 12 months while the duration of exposure in rodents for a food-use chemical should be at

least 24 months. The duration of exposure to non-rodents should be 12 months. J-MAFF require rodent studies to continue for 18 months (mice) or 24 months (rats).

STUDY OBSERVATIONS

Clinical data:

The animals should be observed daily. A careful clinical examination should be made at least once each week (US-EPA). Special attention must be paid to mortality and tumour development including the time of onset, the degree and duration. Daily examination is necessary to minimize loss of animals. Body-weights should be recorded individually for all animals once a week during the first 13 weeks of treatment and at least once every 4 weeks thereafter.

Food or water consumption should be determined weekly during the first 13 weeks and then at approximately monthly intervals.

Ophthalmological examination should be made prior to administration of the test material and at the end of the study, preferably in all animals, but at least in the high-dose and controls. If changes are detected all animals should be examined (US-EPA, J-MAFF).

Haematology:

Haematological examinations should be performed at approximately 6 months intervals during and at the end of the study from 10 rodents/sex of all groups and from all non-rodents (US-EPA), or at 3, 6 and 12 months (OECD). A differential blood cell count should be performed on samples of the animals in the highest dose group and the controls. If necessary, it should be performed on samples of the next-lower dose group and also on sick animals. If haematological effects were noted in the subchronic toxicity study, haematology should be performed at 3, 6, 12, 18 and 24 months for a two-year study (this blood sampling schedule is also recommended for the testing of agrochemicals) and at 3, 6 and 12 months for a one-year study (US-EPA).

Clinical chemistry:

Assays should be conducted at approximately 6-month intervals and at termination (OECD) or at the beginning, middle and at the end of the study (US-EPA) from all non-rodents and from 10 rodents/sex of all groups. In addition, a pre-test sample should be collected from non-rodents (OECD). There are differences between the guidelines concerning the number of parameters investigated.

Urinalysis:

Urine samples taken from all non-rodents and from 10 rodents/sex of all groups are analysed at the same interval as haematological examinations (OECD, US-EPA, J-MAFF).

Post-mortem examinations:

Complete gross pathology should be carried out on all animals including those which died or were killed during the study. Organs and tissues of all animals should be preserved for microscopic examination. Weight determinations of selected organs (at least liver, kidneys, brain and testes) are requested by US-EPA(FIFRA) and J-MAFF.

These organs taken from at least 10 rodents/sex per group and of all non-rodents should be weighed.

Histopathology:

Should be performed in all non-rodents on all preserved organs and tissues of the animals that died or were killed during the study and on the target organs and on all gross lesions in all animals (OECD, US-EPA, J-MAFF; for rodent studies all preserved organs and tissues from the highest dose group and controls together with target organs and on all gross lesions in all animals should be examined (OECD, US-EPA, J-MAFF).

If excessive early deaths or other substantial alterations occur in the highest dose group, complete histopathology should be performed on the next dose level.

B.4 CHRONIC TOXICITY STUDIES: PHARMACEUTICALS

(EEC, US-FDA, J-MHW)

STUDY DESIGN

Animal species:

Two species, one rodent (usually rat) and one non-rodent (dog or monkey) are recommended (EEC, US-FDA, J-MHW).

Group size:

At least 10 - 25 rodents/sex/group and 3-4 non-rodents/sex/group are required for each dose level and for the control. The numbers should be increased if interim killings or a follow-up period are planned.

Dose levels:

At least 3 dose levels and a control are recommended.

The highest dose should induce some toxicity/toxic effects or should bring harmful effects to light without causing excessive lethality.

The intermediate dose should be established in a mid-range between the high and low doses.

The lowest dose should not produce any evidence of toxicity.

The selection of the dose levels should take into account data from preceding toxicity studies (sub-chronic study). The lowest dose should relate to the proposed daily therapeutic dose.

Duration of treatment:

This depends on the duration of use in man. For unlimited use at least 6 - 12 months studies with rodents and 12 months studies with non-rodents are recommended.

STUDY OBSERVATIONS

Clinical observations:

Should be performed at least once daily in all animals. Careful observations should be made to detect onset and progression of all toxic effects including suspected tumours as well as to minimise loss due to disease, autolysis or cannibalism.

Clinical signs, including neurological and ocular changes as well as mortality, should be recorded for all animals.

Body-weight and food consumption should be recorded weekly (EEC) or on a weekly basis for the first 3 months, thereafter once a month (J-MHW).

Eye examinations (direct and/or indirect ophthalmoscopy) should be performed before the start and at the end of treatment at least in control and high-dose animals. If changes are detected, all animals should be examined.

Haematology:

Haematological examination should be performed at 3 and 6 months and thereafter at approximately 6-month intervals and at termination of the study on blood samples collected from at least 10 rats/sex of all groups and from all non-rodents. If possible, samples should be from the same rats at each interval. From non-rodents samples should also be collected before the start of treatment. A differential blood count is performed on samples from the animals of the high-dose group and the controls. If indicated, counts should be performed on the next-lower group(s) as well (EEC).

Clinical chemistry:

At approximately 6-monthly intervals and at termination, blood samples should be collected for clinical chemistry from at least 10 rats/sex/group and from all non-rodents, using if possible the same rats at each interval. From non-rodents samples should be collected before start of treatment.

Urinalysis:

Urine samples should be collected for analysis from at least 10 rats/sex/group and from all non-rodents at the same intervals as haematological examination.

Gross necropsy:

Should be performed on all animals, including those which died or were killed during the study. Organs and tissues of all animals and all visible lesions and tumours or lesions suspected of being tumours should be preserved. An attempt should be made to correlate gross observations with the microscopic findings.

The weights of the following organs should be recorded:
brain, pituitary, liver, kidneys, adrenals, heart, spleen, thyroids, thymus, testes, prostate, ovaries, uterus.

Histopathology:

Should be performed on all grossly-visible changes, tumours or lesions suspected of being tumours in any organ in all groups, and on the organs and tissues of all animals which died or were killed during the study. Further histopathology should be carried out on all animals of the control and high dose group.

Organs or tissues showing abnormalities caused, or possibly caused, by the test substance in the high-dose group should be examined histopathologically in the other (mid- and low-) dose groups.

If survival of the high dose group is substantially less than the control, or if there is evidence of induction of effects in the high-dose group which might affect a toxic response, then the next-lower dose group should be examined as described above.

APPENDIX C. GLOSSARY

GENOTOXICITY

A broad term which refers to potentially harmful effects on genetic material, which may be mediated directly or indirectly, and which are not necessarily associated with mutagenicity. Thus, genotoxicity tests include tests which provide an indication of induced damage to DNA (but not direct evidence of mutation) via effects such as unscheduled DNA synthesis (UDS), sister chromatid exchange (SCE) or mitotic recombination, as well as tests for mutagenicity.

GENOTOXIC COMPOUNDS

Compounds showing clear indication of mutagenic or DNA-damaging effects in validated test systems. Due to a relatively close correlation between mutagenic and carcinogenic effects in humans as well as animal experiments it is generally assumed that genotoxic compounds are potential carcinogens.

GENOTOXIC CARCINOGENS

Those genotoxic compounds which have been shown to induce (malignant) tumours, increase significantly the spontaneous incidence of tumours or decrease the latency period of tumour development by a presumed or proven genotoxic mechanism.

HAZARD

According to the EEC: the inherent capacity of a substance to cause adverse effects.

HAZARD ASSESSMENT

According to the EEC: the assessment of the potential of a substance to cause harm to the target groups exposed to the substance. An environmental hazard assessment uses information on environmental exposure (environmental compartments of concern, quantities) and effects data (with reference to the environmental compartment of concern) and is normally expressed by a comparison of the (predicted) environmental concentration with the (predicted) no-effect concentration for test species or ecosystems. Similarly a human hazard assessment compares the projected human exposure with the no-effect concentrations for species used in toxicity testing.

HAZARD IDENTIFICATION

First stage in the identification of properties of a substance which may be of toxicological or environmental concern.

METABOLIC BREAK POINT

The tissue concentration at which the rate of metabolism and/or the nature of metabolites shows a step change indicating saturation of primary metabolism and/or initiation of an alternative pathway.

MUTAGENICITY

The induction of permanent transmissible changes (mutations) in the amount or structure of the genetic material of cells/organisms. The mutation may involve a single gene or gene segment, a block of genes or whole chromosomes.

Effects on whole chromosomes may be structural and/or numerical. Mutations in somatic cells may be transferred to daughter cells. Mutations in the germ cells of sexually-reproducing organisms may be transmitted to the offspring.

NON-GENOTOXIC CARCINOGENS

Those compounds which increase the incidence of (malignant) tumours (or shorten latency period) for which there is no indication of genotoxicity in validated tests. This tumour increase is often confined to the highest dose tested (MTD) and often accompanied or preceded by non-neoplastic functional and/or morphological changes in the target organ.

NON-GENOTOXIC COMPOUNDS

Those compounds for which there are no indications of mutagenic or DNA-damaging effects in validated test systems.

PHARMACOKINETICS

Optimisation of the dosage of therapeutic agents required for preclinical evaluation of pharmaceutical materials. (qv: toxicokinetics).

RISK

According to the EEC definition, risk is the measured or estimated probability of a toxic response following exposure to a hazardous chemical. It may be expressed semi-quantitatively, for example as high, medium or low, or quantitatively in absolute terms as the probability of occurrence of an effect.

RISK ASSESSMENT

The risk assessment estimates the probability that a substance will cause adverse effects as a result of the presence (at a given concentration) of that substance in the environment. The term risk assessment is often confused with the term hazard assessment; it is frequently used as a comprehensive term to cover any kind of adverse effect evaluation of substances. As a risk usually is expressed as the probability of the occurrence of an adverse effect, the term "risk assessment" should not be used if no probabilities are calculated.

SAFETY FACTOR

Synonymous with uncertainty factor (UF) used by the US-EPA, this is the factor applied to a no- effect level to calculate an acceptable daily intake (ADI) and which allows for uncertainties in extrapolation to man from experimental studies in animals. The magnitude of the safety factor may increase with allowance for factors such as inter- and intra-species variation, extrapolation from studies of less than chronic duration, availability of experimental data from only one laboratory species and use of a LOAEL (lowest observable adverse effect level) rather than a NOAEL (no observable adverse effect level).

TOXICOKINETICS

The extent and time course of adsorption, distribution, metabolism and excretion of a foreign compound in toxicological studies. This includes an assessment of systemic exposure to chemicals, but also in the case of incidental exposures in accidents or multiple low exposures as experienced in an occupational situation (qv: pharmacokinetics).

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